Environmental Research Section A **87,** 69–80 (2001)
doi:10.1006/enrs.2001.4288, available online at http://www.idealibrary.com on IDEAL®

Cadmium Absorption in Women Fed Processed Edible Sunflower Kernels Labeled with a Stable Isotope of Cadmium, ^{113}Cd[1]

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Received November 14, 2000

The apparent fractional absorption of cadmium (Cd) from sunflower kernels (SFK) was determined in women volunteers by using kernels labeled with a stable isotope of Cd (^{113}Cd) by injecting it into the flowering head. Fourteen women who were between the ages of 30 and 70 years, who did not use tobacco products, who were in good health, and who had been consuming a self-selected diet low in Cd content participated in the study. The volunteers were fed a breakfast composed primarily of cereal, milk, and fruit juice. The breakfast also contained a portion of ^{113}Cd-labeled SFK processed into a buttery spread. Each volunteer collected individual stool samples for 21 days beginning immediately after they had consumed the labeled kernels. The total amounts of Cd and ^{113}Cd excreted in each stool were determined by isotope dilution inductively coupled plasma mass spectrometry. Mean fecal Cd excretion was 14.1 ± 4.1 μg/day and mean ^{113}Cd absorption was 10.6 ± 4.4%. In agreement with previous studies, no significant ($P > 0.3$) correlation between Cd absorption and serum ferritin concentrations was found in women whose serum ferritin concentrations were >25 ng/mL. These data suggest that the availability of Cd from highly processed sunflower kernels to humans is similar to that reported for other types of food.

Key Words: **apparent absorption; cadmium; stable isotope; sunflower kernels; women.**

INTRODUCTION

Cadmium (Cd) can be toxic if consumed in large amounts. The biological half-life of Cd in humans is approximately 7 years in the liver and 17 years in the kidney (Nomiyama, 1980). Therefore, a continuous intake of low amounts of Cd over long periods could cause Cd accumulation in the kidney and liver, and lead to organ damage. Food is the major source of Cd intake in the United States with the highest concentrations occurring in such foods as liver and kidney; cereals, including corn, oats, and wheat; vegetables including kale, lettuce, spinach, and culinary herbs (Tahvonen, 1996). The range of daily intake of dietary Cd of adult men and women in the United States has been estimated between 10 and 20 μg/day, or 70 to 140 μg/week (Gartrell *et al.*, 1986; Pennington *et al.*, 1986). Global dietary Cd intakes of 10 to 40 μg/day have been estimated for nonpolluted areas.

Because of inherent genetic and physiological characteristics, some food crops, including sunflowers, take up Cd from the soil in which they are grown, and some of the Cd is deposited in the kernels. As a result, sunflower kernels (SFK) tend to have higher natural concentrations of Cd than most other grains, even when grown in uncontaminated soils (Li *et al.*, 1995). The concentration of Cd in SFK

[1]This study was funded by a grant from the International Lead Zinc Research Organization (ILZRO) through Project Number CH-78/171-2. Volunteers were admitted to the study at the Grand Forks Human Nutrition Research Center (GFHNRC) after having been informed of its purpose and any associated risks and benefits, and signed an informed consent statement (on file at the GFHNRC). This study was approved by the Institutional Review Board of the University of North Dakota and the Human Studies Committee of the U.S. Department of Agriculture, Agriculture Research Service, in accordance with U.S. Title 21 Code of Federal Regulations Part 50, Protection of Human Subjects. Informed consent and experimental procedures were consistent with the Declaration of Helsinki regarding the use of human subjects. U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

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0013-9351/01 $35.00

ranges from 0.2 to 2.5 μg/g fresh weight, depending on the genotype, the weather conditions, and the local soil conditions in the area where the sunflowers are grown (Chaney *et al.*, 1996; Li *et al.*, 1995). Chemical analyses of 16 different lots of SFK from the 1993 crop grown in the Northern Great Plains region of North Dakota and Minnesota, U.S.A., showed an average ± SD of 0.47 ± 0.15 μg Cd/g of fresh kernel (range, 0.26–0.74 μg/g). Although SFK are not a staple food item, because of local customs and individual food choices, it is possible that certain populations could increase their intake of Cd by consuming high amount of SFK.

The World Health Organization (WHO) guidelines recommend a provisional tolerable weekly intake (PTWI) of 490 μg of Cd for a 70-kg person (Walker and Herrman, 2000). Based on the SFK-Cd concentrations cited above, an average daily consumption of 1 oz (28.35 g) of SFK could increase the total intake of Cd from an average of ~100 to ~200 μg/week. Although this is only 40% of the PTWI, higher intakes of kernels or a higher Cd content could increase the average intake and increase the Cd burden of the consumer, if the Cd were indeed available for absorption.

Cadmium in food must be absorbed and retained in order to increase body burden. Compared with other trace elements, Cd absorption from foods such as kidney and liver, oatmeal, and crab, is low. In most studies, the range is from 0.7 to 15% in humans (McLellan *et al.*, 1978; Newton *et al.*, 1984; Rahola *et al.*, 1973; Shaikh and Smith, 1980), and from 0.3 t5o 25% in animals (Kello and Kostial, 1977; Kostial *et al.*, 1978; Reeves *et al.*, 1994). Most of these studies were conducted with food extrinsically labeled with radioactive Cd, as ^{109}Cd or ^{115m}Cd (Table 1, Absorption). The amount of dietary Cd absorbed depends largely on the mineral composition of the diet, the sex, body iron stores, and the age of the consumer, and possibly on the speciation of Cd in the diet (Fox, 1988; Groten and van Bladeren, 1994).

Studies to determine the availability of Cd from SFK in animals and humans are limited. Cd concentrations were higher in the organs of Japanese quail fed diets containing the seed pomace of sunflowers grown on Cd-laden sludge-amended soils than in birds fed similar diets with sunflowers grown on control soils (Stoewsand *et al.*, 1986). Cd absorption was not measured in this study. Previously, we found that rats absorbed 20% less Cd from diets containing 20% ground SFK extrinsically labeled with ^{109}Cd than from diets not containing SFK (Reeves *et al.*, 1994). One study in humans showed that individuals who reported consuming greater than 1 oz (28.35 g) of SFK per day for more than a year were no more likely to have a higher body burden of Cd, as assessed by blood Cd concentration and urinary Cd excretion, than those who consumed a smaller amount of kernels. However, absorption was not determined in this study (Reeves and Vanderpool, 1997).

Because of the paucity of information about Cd availability from SFK in humans, the present study was designed to determine the absorption (intake minus fecal excretion) of Cd from kernels labeled with ^{113}Cd, a stable isotope of Cd. Data from this type of study can aid in the calculation of exposure and help better define the intake of cadmium for a population.

METHODS AND MATERIALS

Volunteers

Women, aged 30 to 70 years, were recruited from the Red River Valley Region of North Dakota and Minnesota. Each volunteer had previously been consuming a diet low in Cd, i.e., low intakes of SFK, oysters, clams, and organ meats, and did not use tobacco products. They had no significant health conditions, and they were not taking prescription medications thought to affect Cd absorption.

At the beginning of the study, the volunteers were consuming a variety of self-selected foods, which they continued to consume throughout the study. The volunteers were accepted into the study if they were in good health and did not have physiological conditions that might influence Cd absorption. They were accepted only if they had hemoglobin concentrations greater or equal to 120 g/L, serum C-reactive protein (CRP) concentrations of less than 5 mg/L, serum ferritin concentrations greater than 25 ng/mL, and were not pregnant. Table 2 lists the characteristics and blood chemistry values of the volunteers at the beginning of the study.

Volunteers were admitted to the study after having been informed of its purpose and any associated risks and benefits, and had signed an informed consent statement. The institutional Review Board of the University of North Dakota and the Human Studies Committee of the United States Department of Agriculture, Agriculture Research Service, approved the study. Informed consent and experimental procedures were considered with the Declaration of Helsinki regarding the use of human subjects.

TABLE 1
Literature Values for Cd Balance and Absorption from Various Food Sources Fed to Human Volunteers

Reference, year	Dose (μCi)	Label	Label type	Absorption (%)	Serum ferritin (ng/mL)	*n*	Meal
			Absorption				
Rahola *et al.*, 1973	Unknown[a]	^{115m}Cd	—[b]	4.7–7.0	—	5	Beef kidney cortex[c]
Flanagan *et al.*, 1978	25[d]	$^{115m}CdCl_2$	Extrinsic	8.9 ± 2.0	<20	10	Rolled oats and milk
	—	—	—	2.3 ± 0.3	> 23	12	Rolled oats and milk
McLellan *et al.*, 1978	5[e]	$^{115m}CdCl_2$	Extrinsic	4.6 ± 4.0	—	14	Rolled oats and milk
Shaikh and Smith, 1980	5–10[f]	^{115m}Cd or ^{109}Cd	Extrinsic	2.5 ± 1.0	>10	7 men	Beef and milk[c]
	—	—	—	3.0 ± 0.1	>10	3 women	Beef and milk[c]
	—	—	—	6.1	<10	2 women	Beef and milk[c]
Newton *et al.*, 1984	30–100[g]	^{115m}Cd	Intrinsic	2.7 ± 0.9	62 ± 38[h]	7 men	Crab hepatopancreas

Reference, year	Intake (μg/day)	Urine Cd (μg/day)	Fecal Cd (μg/day)	Balance (μg)	Serum ferritin (ng/mL)	Diet	Sex (*n*)
				Balance			
Bunker *et al.*, 1984	8.6	0.4	9.9	−15	77.0	Self-selected	Men and Women (23)
Mueller *et al.*, 1993	11.92	0.44	10.9	+ 0.58	—[b]	—	Men (—)
	10.29	0.53	9.5	+ 0.26	31[i]	—	Women (—)
Berglund *et al.*, 1994	11 ± 4.2	0.17 ± 0.11	11 ± 4.5	0.0	31 ± 30[j]	Mixed	Women (34)
	16 ± 7.1	0.19 ± 0.18	16 ± 7.5	0.0	26 ± 26[k]	High fiber	Women (23)
Reeves *et al.*, 1997	30–68[l]	—	24–34	—	11–167	Self-selected	Men and Women (125)

[a]100 μg ^{NA}Cd.
[b]Indicates a blank space.
[c]Beef kidney cortex homogenate.
[d]25 μg ^{NA}Cd.
[e]22–29 μg ^{NA}Cd.
[f]50 μg ^{NA}Cd.
[g]kBq; 61 ± 53 μg ^{NA}Cd.
[h]One volunteer <20 ng/mL.
[i]Range 11–86.
[j]Range 3–124.
[k]Range 3–38.
[l]Values were calculated from a database similar to GRAND and adjusted based on selected analyses of total diet collections.

Experimental Design

Fourteen women were admitted into the study and continued to live in their homes and select their own diets except for a single breakfast and lunch eaten at the Center. The study began with a 7-day baseline fecal collection. After the last collection, and at approximately 6:00 AM, each volunteer consumed a breakfast consisting of the foods listed in Table 3 (breakfast). This breakfast contained SFK labeled with the stable isotope ^{113}Cd (see following sections). The entire portion of each ingredient was consumed, the containers were rinsed with deionized water, and the water consumed. The volunteers consumed only water until noon the same day, at which time they were asked to eat a lunch consisting of the ingredients listed in Table 3 (lunch). This meal did not contain labeled Cd. The volunteers then returned to their self-selected diets.

Each volunteer provided fecal collections consisting of individual bowel movements for a period of 21 days after first receiving the labeled Cd meal. Individual stool samples were analyzed for both natural abundance and isotopic enrichment of Cd. Blood and urine were also collected during the study, and various blood chemistry analyses were performed

TABLE 2
Physical Characteristics and Blood Chemistry of the Volunteer at the Beginning of the Study[a]

Volunteer characteristics ($n = 14$)		
Height, cm	161 ± 8	Weight, kg 72 ± 16
BMI,[c] kg/m^2	27 ± 6	Age, years 52 ± 13
Blood chemistry		Normal values[b]
C-reactive protein, mg/L	0.41 ± 0.23	<5
Eosinophils, %	2.8 ± 1.6	1-3
Hemoglobin, g/L	134 ± 4	120-150
Hematocrit, %	40 ± 2	36-46
Mean corpuscular		
Hemoglobin, pg	30 ± 2	26-34
Volume, fL	90 ± 5	82-98
White blood count, $\times 10^9$/L	6.0 ± 1.4	4.4-10
Lymphocytes, %	31 ± 7	25-35
Monocytes, %	8 ± 2	2-6[d]
Neutrophils, %	57 ± 8	50-70
Platelet count, $\times 10^9$/L	262 ± 42	150-400
Red blood cells, $\times 10^{12}$/L	4.4 ± 0.4	4.6-6.0
Serum ferritin, ng/mL	68 (38, 122)[e]	10-125

[a]Mean ± SD.
[b]Normal clinical range for adults (Kee, 1995).
[c]Body mass Index.
[d]Normal range at the Grand Forks Human Nutrition Research Center is 5%-12%.
[e]Data were log transformed. Means have been transformed back to the original scale. Numbers in parentheses represent (mean − 1 SD, mean + 1 SD).

immediately. The remaining blood samples and urine were frozen for future analyses; however, because of a devastating flood shortly after sample collections were completed, the blood and urine samples thawed and sat at room temperatures for nearly 2 months. Consequently, neither set of these samples was evaluated for Cd content.

During the first week of the study, volunteers recorded all foods and beverages consumed in a 3-day food diary. The 3 days consisted of Thursday through Saturday to cover possible differences in eating patterns between weekdays and weekends. On completion of the diary, a dietitian interviewed each of the volunteers to ensure completeness and accuracy of their recorded information. The nutrient content of foods in the diary was calculated by using the Grand Forks Research Analysis of Nutrient Data (GRAND) nutrient database maintained by the USDA, ARS, Grand Forks Human Nutrition Research Center (Grand Forks, ND). Values for the GRAND database are derived from the USDA, ARS, Nutrient Database for Standard Reference, Release 13 (Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp), and supplemented with other reliable sources and actual in-house analyses. The database also was supplemented with Cd values from the analysis of over 300 foods obtained from the FDA Total Diet Study, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC (http://www.cfsan.fda.gov/~comm/tds-toc.html). The SFK used in the present study was analyzed for Cd content, and those values were entered into the database and used to estimate Cd in the total diet.

Production and Labeling of SFK

Individual sunflower plants (*Helianthus annuus* L.; Agway Royal Hybrid 2141; Agway, Inc., Grandin, ND)[4] were grown in 15-gallon plastic pots (one plant/pot) containing 11 gallons of soil prepared from a mixture of 50% Fargo clay (Li *et al.*, 1994) and 50% Sunshine Mix 1 (sphagnum peat moss and horticultural pearlite), a general potting soil. Each pot was supplemented with 100 g of Osmocote slow release fertilizer (14-14-14) and 50 g of Micromax micronutrients (Grace Sierra, Milipitas, CA). When the plants had approached maturity and had begun to initiate flowering, the stem of each flower head was injected with a buffered solution containing ^{113}Cd. This solution was prepared by dissolving 25 mg of ^{113}Cd metal (95.2 ± 0.3 at.%; NMR Advanced Materials Inc., New York, NY) in a slight excess of 22% HNO_3. After gentle heating of the mixture to dissolve the metal, the solution was heated to dryness. The residue was dissolved in 1 mL of 10 M HCl and taken to dryness. This process was repeated three times. The $^{113}CdCl_2$ was then dissolved and diluted to 75 mL with a citrate–phosphate buffer (23.3 mL of 0.1 M citric acid and 26.7 mL of 0.2 M dibasic sodium phosphate) (Gomori, 1955). The resulting 3.0 mM ^{113}Cd solution was stored in a polypropylene bottle and refrigerated when not in use. The sunflowers were injected with the Cd-containing buffer solution at the greenhouse temperature of approximately 27°C. Injections were made twice weekly for 5 weeks by using a 26-gauge, 0.5-in. needle on a 1.0-mL plastic tuberculin syringe. The labeling sequence consisted of injecting 0.1-mL increments of the ^{113}Cd solution at four separate

[4]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

TABLE 3

Food Items in the Breakfast and Lunch Meals and Calculated Values for Selected Nutrients

Food item	Amount (g/serving)	Calories (Kcal)	Protein (g)	Fat (g)	CHO[a] (g)	Phytate[b] (mg)	Ca (mg)	Fe (mg)	Zn (mg)
Breakfast									
Apple juice	180.00	84.60	0.11	0.20	21.02	0.00	13.91	0.666	0.036
Rice Krispies	25.00	99.0	1.60	0.10	22.15	51.75	4.50	0.625	0.415
Milk, 2%	195.00	96.86	6.49	3.74	9.36	0.00	218.40	0.098	0.722
Sugar	5.00	19.35	0.00	0.00	5.00	0.00	0.05	0.003	0.002
Bread, white	30.00	80.10	2.46	1.08	14.85	20.70	32.40	0.909	0.186
Jelly, grape	15.00	40.65	0.06	0.02	10.62	0.00	1.18	0.039	0.009
SFK	18.25	106.22	3.53	9.08	4.39	293.26	15.22	1.049	1.025
Salt, table	0.12	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.00
Margarine	9.02	64.83	0.09	7.27	0.09	0.00	1.24	0.00	0.006
Water	200.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Meal totals	677.39	591.60	14.33	21.49	87.47	365.71	287.23	3.389	2.401
Lunch									
Grape juice	180.00	109.80	1.01	0.14	26.93	0.00	16.45	0.432	0.090
Hamburger bun	40.00	114.40	3.40	2.04	20.12	32.80	42.40	1.308	0.038
Turkey	50.00	78.50	14.95	1.61	0.00	0.00	9.50	0.675	1.02
Cheese, cheddar	25.00	100.64	6.22	8.28	0.32	0.00	166.25	0.170	0.848
Lettuce leaf	10.00	1.30	0.10	0.02	0.21	0.00	0.62	0.015	0.008
Mayonnaise	15.00	107.52	0.16	11.91	0.41	0.00	0.81	0.038	0.017
Potato chips	30.00	160.80	2.10	10.38	15.87	58.80	7.20	0.489	0.327
Vanilla wafer	30.00	141.90	1.29	5.82	21.30	11.10	7.50	0.663	0.099
Water	200.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Meal totals	580.00	814.9	29.24	40.21	85.18	102.70	250.73	3.79	2.447

[a]Total carbohydrate.
[b]Harland and Oberleas, 1987.

sites on the stem-head junction of each plant. After the final injection and the plants had grown to maturity, the heads were allowed to dry while still attached to the plant. The sunflower heads were harvested, and the kernels were manually removed from the shells and dried for 2 days at 40°C on a hot plate. The kernels were stored at −20°C until further processing was done. Analyses showed that of the 25 mg of ^{113}Cd isotope injected into the 15 sunflower plants, 0.30 mg were recovered in 632 g of kernels, for an isotopic incorporation of 1.2%.

Preparation of SFK Dose

Labeled (enriched) SFK were placed on a cookie sheet in a single layer and roasted at 149°C for 15 min. After the roasted kernels were cooled, they were transferred to a food processor and blended into a smooth paste. To each 18.2 g portion of the blended kernels, 120 mg of salt and 9.0 g of margarine were added. This mixture was blended again and labeled as "sunbutter" (Johnson *et al.*, 1988). Sunbutter that was not enriched with ^{113}Cd, i.e., contained only natural abundance Cd, was prepared from raw SFK (EVON'S, John B. Sanfilippo & Sons, Inc., Elk Grove Village, IL) purchased at a local supermarket. They were prepared in the same manner as described above.

The individual dose of sunbutter was prepared for each volunteer by combining 5.2 g of ^{113}Cd-enriched sunbutter and 22.2 g of unenriched or natural abundance Cd sunbutter. The prepared doses were stored in wide-mouth glass containers with polypropylene caps and Teflon fluorocarbon resin/silicone septa for purging with nitrogen. Prior to use, the jars were washed in a commercial acid/soap-containing solution (Radiac; Atomic Products Corp., Shirley, NY), soaked in 6 N HNO_3 and rinsed with quartz subboiling distilled (QSBD) water. The caps were washed in Radiac and rinsed in QSBD water. After the sunbutter was added to the jars, they were purged with nitrogen and stored at −80°C until used.

Instrument and Calculations

Cadmium isotope ratios were measured by using isotopic dilution with a VG PlasmaQuad 2+ inductively coupled plasma mass spectrometer (ICPMS; VG Elemental, Winsford, UK) with no significant instrument modifications. Typical operating

conditions were previously described by Vanderpool *et al.*, (1994). Isotope dilution ICPMS (IDICPMS) spike solutions were prepared from ^{110}Cd (95.6 $\pm$ 0.3 at.%, NMR Advanced Materials Inc., New York, NY) and stored in Teflon bottles. Isotopically enriched solutions were analyzed by reverse isotope dilution ICPMS. Natural abundance Cd standards were prepared daily from a stock solution (ICP standard solution, Æsar, Ward Hill, MA). A reference isotope, ^{111}Cd, was used in all IDICPMS measurements. All solutions were prepared with QSBD water and mineral acids.

Natural abundance and isotopic Cd enrichment, ^{110}Cd and ^{113}Cd from ICPMS intensities, were calculated as previously described for copper (Buckley *et al.*, 1996). Isotopic ion intensities for SFK, sunbutter, or fecal samples were obtained from the IDICPMS analysis, and were blank and bias corrected before using the TTMR9 Program[5] to calculate tracer/tracee mass ratios (TTMR = TR/TE) for subsequent isotopic enrichment. Natural abundance isotopic compositions for Cd were obtained from a standard source (Rosmani and Taylor, 1998), while enriched isotopic compositions were taken from data sheets supplied by NMR Advanced Materials, Inc., NY. Total fecal ^{113}Cd and natural abundance Cd from each volunteer was calculated by adjusting for sample aliquot and total fecal sample, and then summing over the entire experiment. Cadmium absorption was calculated by subtracting the amount of ^{113}Cd excreted in the feces, in excess of natural abundance, from the amount fed in the breakfast meal. This amount was then divided by the amount of ^{113}Cd in the meal. The resulting value is referred to as apparent absoption because it does not account for endogenous secretions of Cd into the gut lumen that might be subsequently excreted in the feces (Johnson, 1982; Johnson *et al.*, 1988). However, endogenous excretion of Cd is less than 0.1% of the dose and would not influence fecal Cd concentration (Nordberg *et al.*, 1985).

Sample Analysis

Two different techniques were used for sample preparations in this study. Sunflower kernels and sunbutter sample were digested and the Cd extracted with an ammonium pyrrolidine dithiocarbamate (APDC, Reagent grade, wt/vol, Aldrich Chemicals, Milwaukee, WI) procedure (Bunker *et al.*, 1984). Fecal samples were digested and the Cd was extracted by using a sodium diethyldithiocarbamate (NaDDC) procedure (Vanderpool and Buckley, 1999). Validation studies indicated that APDC chelation was suitable for the analysis of Cd in sunflower kernels and sunbutter because natural abundance isotope ratios could be measured at 1.5% error (data not shown). However, APDC chelation was not suitable for extracting Cd from human fecal samples (data not shown) and a NaDDC extraction method was significantly modified to extract Cd from this material (Vanderpool and Buckley, 1999). The APDC procedure was slightly modified as described below.

For the extraction of sunflower kernels or sunbutter samples, a 10% ammonium citrate solution (Æsar, ACS grade, wt/vol) was prepared and purified by storing it over Chelex-100 ion-exchange resin. The reagent was stored in a plastic bottle at 4°C and prepared fresh monthly. Before use, the reagent was warmed to room temperature and filtered through acid washed glass wool in a glass funnel to remove the ion-exchange resin. A 1% (wt/vol) APDC solution was prepared fresh daily and washed three times with methylisobutyl ketone (MIBK, Reagent grade, Aldrich Chemicals) in an acid washed separatory funnel with a Teflon-plug stopcock. A 5% potassium hydroxide solution was prepared from 30% potassium hydroxide (Æsar, potassium hydroxide, 99.995% purity).

One-half gram each of SFK and/or sunbutter was spiked with freshly prepared ^{110}Cd and 2 mL of 16 M HNO_3, and then heated on a hot plate at 200°C until dry (about 6 h). The dried samples were covered with watch glasses and placed in a muffle furnace overnight at 460°C. After the residue was cooled, it was dissolved in 1 mL of 16 M QSBD HNO_3, taken to dryness, and placed in the furnace at 460°C for 2 h. The residue was dissolved in a minimum of 1 mL of 1 M HCl, 1.5 mL of 10% ammonium citrate and the pH adjusted to 4.3 with 5% KOH. The solution was then transferred to a 252 Sedipet bulb (SAMCO transfer pipets, Saint-Amand MFG., San Fernando, CA) with 1.5 mL of 1% APDC and mixed with a vortex mixer. Cadmium was then extracted from the aqueous phase by adding 1.5 mL of MIBK and again mixing with a vortex mixer. After the phases separated, the aqueous phase was discarded and the MIBK/APDC solution was washed twice with 4 mL of QSBD water. The organic phase Cd was back extracted into 4 mL of 2% HNO_3 and the aqueous phase saved. The aqueous phase was taken to dryness in a dry bath at 80°C. When ready for IDICPMS analysis, the samples were dissolved in 8% HNO_3 and diluted to volume with QSBD water to give a 2% HNO_3 solution.

[5]Budac, J. J., 1992. TTMR9 Ver 1.0. Agriculture Canada Research Station, Agassiz, British Columbia, Canada.

To determine detection limits during analytical method development, five separately processed procedural blanks were measured (vs a 5 ng/mL standard) per day for 13 different days ($n = 65$) over a 3-month period. Detection limits, defined as three times the procedural blank, were: 0.16 ng/mL for the ^{110}Cd spike, ^{111}Cd reference, and the ^{113}Cd label.

Cadmium was measured in sunflower kernels, sunbutter, a pooled breakfast, and a pooled lunch by IDICPMS. Other trace elements in the above foods were obtained by using a Jarrel-Ash 1140 inductively coupled plasma optical emission spectrometer (ICPOES) with standard analysis conditions. Blood chemistry data were obtained by using a CellDyne 3500 System (Abbott Laboratories, Abbott Park, IL). Serum C-reactive protein (CRP) was measured by using a Behring Nephleometer 100 Analyzer (Behring Diagnostics, Inc., Westwood, MA). Both C-reactive protein and ferritin will increase because of inflammation. Thus, C-reactive protein was determined to rule out high ferritin values caused by inflammation unrelated to iron status. Serum ferritin was determined by using a commercial kit (Abbott Laboratories, Diagnostic Division, Abbott Park, IL).

Statistical Analysis

Statistical analysis was performed by using the nonparametric Kruskal-Wallis test for differences between age groups (Hollander and Wolfe, 1999) (SAS Institute Inc., Cary, NC).[6] To analyze for serum ferritin effects on Cd absorption, a regression of percentage Cd absorption vs natural log (serum ferritin) was conducted.

RESULTS AND DISCUSSION

To measure the absorption of trace elements in humans, the element often is added to foods as an inorganic salt. This technique is referred to as extrinsic labeling. Alternatively, the label is incorporated into the food by a physiological route and is termed intrinsic labeling. The absorption of an intrinsic label has physiological implications because it should have similar chemical characteristics in the food as the naturally occurring element. Therefore, absorption values obtained in this manner should be less ambiguous than those obtained by using an extrinsic label. In the present study, we labeled sunflower kernels with ^{113}Cd by injecting the label into the flower stem of the growing plant. After the seeds had incorporated the label and matured, they were highly processed into a paste, which we called sunbutter, and fed to female human volunteers as part of a standard breakfast-type meal. Absorption was determined by measuring the excretion of the label in the feces over a 21-day period. While the form of labeling was not extrinsic, we have no evidence that the chemical environment of the plant-injected label was identical to Cd naturally assimilated through the roots; therefore, we have not defined the label as extrinsic or intrinsic.

Fourteen women completed the study, and as shown in Table 2, all blood chemistry values were within normal ranges, except for percent monocytes, which were slightly above standard values (Kee, 1995), but within the range (5–12%) for normal volunteers around the Grand Forks area.

The breakfast and lunch that were fed to the volunteers were designed to contain foods normally consumed by individuals in the Grand Forks, ND area, and to assure that mineral intakes did not exceed the Recommended Dietary Allowances (RDA) (National Research Council, 1989) or the Dietary Reference Intakes (DRI) (Institute of Medicine, 1998). This was especially important for mineral elements such as iron (Fe), zinc (Zn), and calcium (Ca), known to influence Cd absorption (Groten and van Bladeren, 1994). Table 3 summarizes the calculated nutrient contents of the meals. The DRI for Ca or the RDA for Fe and Zn supplied by the breakfast and lunch combined were 67%, 48%, and 40%, respectively. While carbohydrate intakes were high, the other nutrients represented only about one-third of the RDA. We also estimated the phytate content of the breakfast and lunch because phytate has been implicated in Cd absorption (Harland and Oberleas, 1987). However, because the values are estimates, no conclusions can be drawn about their effects on Cd absorption in this study.

The sunbutter served with the breakfast was prepared from two different sources of SFK. One source was obtained from a local supermarket and was not isotopically enriched, while the other was grown in a greenhouse and isotopically enriched with ^{113}Cd. The mineral compositions of the different sources of SFK were analyzed by ICPOES and IDICPMS, and the results shown in Table 4. There was significantly less Ca ($P < 0.01$), but more ($P < 0.01$) magnesium (Mg), manganese (Mn), phosphorus (P), Zn, and Cd in the SFK grown in the greenhouse than in SFK purchased from the supermarket. Iron was also significantly higher ($P < 0.05$), but copper (Cu) concentrations were not different ($P > 0.05$) in greenhouse kernels as compared with the commercial variety.

[6]SAS Institute Inc., SAS Campus Drive; Cary, NC 27513-2414.

TABLE 4
Analyzed Mineral Composition of SFK Enriched and Unenriched with ^{113}Cd

	Unenriched SFK		Enriched SFK	
Element	Analyzed[a]	% of calculated values[b]	Analyzed[a]	% of calculated values[b]
Ca, mg/g	0.682 ± 0.011[c]	97	0.550 ± 0.026	78
Cu, μg/g	11.88 ± 0.23	65	11.42 ± 0.45	62
Fe, μg/g	36.3 ± 2.9[c]	96	41.6 ± 1.6	110
Mg, mg/g	2.40 ± 0.05[c]	186	3.16 ± 0.13	246
Mn, μg/g	15.65 ± 0.79[c]	74	35.9 ± 1.4	170
P, mg/g	5.03 ± 0.10[c]	45	7.75 ± 0.18	67
Zn, μg/g	37.0 ± 1.7[c]	70	66.1 ± 3.1	125
Cd, ng/g	323.8 ± 1.5[c,d]	81	870.5 ± 7.2[d,e]	218

[a]ICPOES analysis, mean ± SD; $n = 4$. Analyzed concurrently with NIST SRM 1548 total diet. Values were found to be within 5% of the certified value for each element, $n = 2$.

[b]Calculated from the GRAND database as dry roasted, unsalted sunflower kernels.

[c]Significantly different from enriched ($P < 0.05$).

[d]IDICPMS analysis, mean ± SD, $n = 6$. Measured NIST SRM 1548a, typical diet, as 33.7 ± 0.4 ng/g DW ($n = 4$) vs the certified value of 35 ± 1.5 ng/g DW for a 3.6% error.

[e]Sum of ^{NA}Cd (388.9 ± 5.5 ng/g) and ^{113}Cd (481.6 ± 4.6 ng/g).

These differences in mineral content were probably caused by differences in soil type, soil pH, chloride content, and other growth conditions (Li *et al.*, 1995). They are listed only to illustrate how growth conditions of the plant can affect its mineral content.

The sunbutter was composed of 19% enriched SFK and 81% unenriched kernels. With this combination, the actual total amount of each mineral consumed because of eating the 18.2g of SFK incorporated into sunbutter is given in Table 5. In addition, several mineral elements that might have affected Cd absorption were measured in the breakfast, the sunbutter fed with the breakfast, and the lunch (Table 5). The total trace element contribution of the sunbutter, breakfast, and lunch also was summarized as percentages of the RDA or DRI, Estimated Safe and Adequate Daily Dietary Intake (ESADDI) (National Research Council, 1989), or as an estimated U.S. daily intake. Totals for Ca and P in the breakfast and lunch were approximately 58% and 73% of the DRI, respectively, and about 70% of the ESADDI for Mn. Total Cd was 100% of the typical U.S. daily intake (20 μg/day) and 70% less than the WHO PTWI of 1 μg/kg body weight/day (Walker and Herrman, 2000). These values closely matched the RDA's and DRI's of diets self-selected from a variety of foods, and suggest that the concentrations of mineral elements were not high or low enough to adversely affect Cd absorption.

Fecal Cd excretion for each volunteer was measured by IDICPMS in individual fecal samples over the whole 28 days of the experiment (7 days before ^{113}Cd was fed and 21 days afterward). Individual values were combined and daily Cd excretion (natural abundance) values are given in Table 6. The mean excretion (± SD) for all volunteers during the 21-day collection period was 14.1 ± 4.1 μg Cd/day ($n = 14$). The estimated Cd intake derived from individual 3-day food diaries by using the GRAND database was 14.4 ± 5.8 μg/day. Daily fecal excretions of Cd, which closely approximate the intake, were consistent with other fecal Cd excretion data reported in the literature (Table 1, Balance). Based on actual values in the references cited, fecal excretion values ranged from 4.4 to 38 μg Cd/day for women consuming various types of food. The difference between calculated and analyzed dietary values for Cd reported in the current study were much smaller than those reported previously for a similar population (Reeves and Vanderpool, 1997). In that report, the calculated intake of Cd was less than 50% of analyzed values of the same diet. The average daily intake of individuals not consuming SFK was about 30 μg/day.

In the present study, the percentage apparent absorption as determined by subtracting the amount of ^{113}Cd excreted in the feces (above natural abundance) from the amount consumed, and dividing by the amount consumed times 100. The values for each volunteer are given in Table 7. Apparent absorption averaged 10.6 ± 4.4% for all volunteers; however, there was wide variability. Two volunteers absorbed less than 5% of the Cd dose, three absorbed between 5, and 10%, and nine absorbed between 10 and 18%.

The gut transit time of an orally administered Cd tracer is highly variable in humans. Typically, the excretion pattern of labeled Cd is composed of two components, a fast phase followed by a slow phase. The fast phase lasts from 3 to 7 days and probably represents mostly unabsorbed Cd, whereas, the slow phase might last for several days and represent not only unabsorbed Cd, but that which had been held up in the enterocytes, but not absorbed into the body. Subsequent natural sloughing and digestion of these cells would release some of the Cd that would then be excreted in the feces (Flanagan *et al.*, 1978; McLellan *et al.*, 1978; Newton *et al.*, 1984; Rahola *et al.*, 1973). Rahola *et al.* (1973) fed human volunteers ^{115m}Cd-labeled beef kidney and reported that the fast excretion rate accounted for about 75% of the dose over the next 3–5 days, while the slow phase

TABLE 5

ICPOES and IDICPMS Analysis of Pooled Samples of Sunbutter, Breakfast, and Lunch[a]

Element	Sunbutter[b]	Breakfast[c]	Lunch	Total	%RDA or DRI	RDA or DRI
Ca, mg	18.0 ± 0.28	234.0 ± 15	230	482	58	1,200[d]
Cu, mg	0.32 ± 0.01	0.12 ± 0.01	0.15	0.59	19	1.5–3.0[e]
Fe, mg	1.02 ± 0.06	2.78 ± 0.22	2.62	6.5	37	15[f]
Mg, mg	69.8 ± 1.3	42.0 ± 2	62	174	39	420[d]
Mn, mg	0.53 ± 0.02	0.54 ± 0.10	0.79	1.9	70	2.0–5.0[e]
P, mg	152.0 ± 0.002	227.0 ± 11	347	576	73	700[d]
Zn, mg	1.16 ± 0.04	1.21 ± 0.09	2.13	4.5	28	12[f]
^{NA}Cd, μg	9.21 ± 0.04[h,i]	0.73 ± 0.10[h]	7.6 ± 1.6[h]	17.54	88	20[g]
^{113}Cd, μg	2.5 ± 0.02[h,i]	—[j]	—	2.5	13	—

[a]Mean ± 1 SD; measured by ICPOES unless otherwise specified; breakfast, $n = 8$; lunch, $n = 2$; analysis concurrent with NIST SRM 1548 total diet and measured within 5% of the certified value for each element, $n = 2$.

[b]One serving = 27.32 g.

[c]Without the contribution of sunbutter.

[d]Dietary Reference Intakes. For Ca = 1200 mg; Mg = 320 mg; P = 700 mg.

[e]Estimated Safe and Adequate Daily Dietary Intake.

[f]Recommended Dietary Allowances.

[g]Estimated U.S. daily intake.

[h]Measured by IDICPMS; measured NIST SRM 1548a, typical Diet, as 33.7 ± 0.4 ng/g DW ($n = 4$) vs the certified value of 35 ± 1.5 ng/g DW for a 3.6% error.

[i]Calculated from the combination of 22.2 g unenriched (see Table 7; 323.8 ± 1.5 ng Cd/g) and 5.2 g enriched sunflower kernel (see Table 7; 388.9 ± 5.5 ng/g ^{NA}Cd and 481.6 ± 4.6 ng/g ^{113}Cd).

[j]Indicates a blank space.

accounted for about 19% of the dose. Another study by Flanagan *et al.*, (1978) found that when human volunteers were fed rolled oats and milk extrinsically labeled with ^{115m}Cd, the rapid excretion phase accounted for about 86% of the dose during the first week, and the slower excretion phase accounted for about 9.4% of the dose during the second week.

In the present study, the label was excreted in 3 to 11 days, with an average of 6.8 ± 2.5 days (Table 7). For most individuals, complete excretion of the isotope occurred in the first 3–4 individuals fecal stools following the dose. Two volunteers excreted the dose in seven enriched stools and two excreted it in only two enriched stools. Volunteers who excreted the dose in seven enriched samples had 10.4 and 14.5% absorption. While a higher apparent absorption could be affected by the distribution of the dose through a number of stools, the highest apparent absorption values (15.9 and 18.3%) were observed in volunteers who excreted most of the Cd isotope in only three stools each. Although transit time of the label through the gut might influence the amount of Cd absorbed, the correlation between Cd absorption and the number of days over which fecal ^{113}Cd enrichment was measurable (Table 7) was not significant (absorption = 0.7 × (days enriched) + 5.8; $r^2 = 0.16$; $P > 0.05$). Thus, the 10.6% absorption reported in this study could be the result of an actual higher absorption rate from SFK or because the slow phase of excretion was extended over several days and resulted in isotope concentrations below the detection limits for this technique, and not all of the unabsorbed Cd was accounted for.

An additional problem arises when the fecal collection period is only a few days long. Crews *et al.*, (2000) fed adult females and infants porridge made from wheat that had been intrinsically labeled with stable ^{106}Cd. By measuring the amount of label excreted in the feces over 4 days in infants and 5 days in adults, they estimated an 18% apparent absorption in infants and 40% in adults. These values are far above those found by others, and surely indicate that longer fecal collections were needed to assure that all of the unabsorbed Cd had cleared the gut.

While trace element excretion is typically quite variable in humans, it is not clear why there was so much variability in apparent Cd absorption among individuals in the current study. Although we tried to select volunteers who were consuming a mixed diet, the variability of components in their usual diet, to which they had adapted prior to the study, could have played a role. Because other trace elements, such as Zn, reduce Cd absorption (Groten and van Bladeren, 1994), the volunteers with low absorption rates might have inadvertently consumed a higher amount of Zn before the test was

TABLE 6

Calculated Dietary Cd Intake from 3-Day Food Diaries and Chemical Analyses of Fecal Cd (Natural Abundance) Excretion

Age (years)	Dietary Cd intake calculated (μg/d)[a]	Fecal Cd output analyzed (μg/d)[b,c]
69.8	12.8	20.4
65.6	15.6	20.2
52.6	11.6	15.0
48.0	23.2	10.0
53.5	12.6	15.3
38.0	22.4	11.4
59.5	14.2	11.8
30.8	16.4	10.4
60.7	15.6	13.3
38.1	7.7	7.9
34.4	10.5	12.1
47.8	18.9	15.9
57.1	4.2	12.4
68.6	18.8	21.3
Mean ± SD 52 ± 13	14.4 ± 5.8	14.1 ± 4.1

[a]Calculated by using the GRAND database.

[b]IDICPMS; $n = 3$ measurements per individual fecal sample for 28 days of sample collection.

[c]An in-house fecal standard (IHFS) was run concurrently by IDICPMS with this study and values obtained were 412 ± 12 ng Cd/g dry weight ($n = 136$). An independent laboratory using ICPOES reported the IHFS Cd concentration to be 471 ± 17 ng/g dry weight, $n = 12$ [NIST tomato leaf standards at 1490 (sample 1) and 1520 (sample 2)]. A second laboratory used ICPMS and reported IHFS Cd concentration at 435 ± 22 ng Cd/g dry, $n = 12$ [NIST tomato leaf standards at 1360 (sample 2) and 1390 (sample 2)].

done. However, food diary records were collected prior to the study to estimate the intake of elements that could have potentially affected Cd absorption. Analyses of these diaries did not show a conspicuously high Zn intake relative to the RDA (Table 8). However, Fe intake was high for women, but not variable, and it is not known whether dietary Fe per se has an effect on Cd absorption in humans. It is well known, however, that iron deficiency increases Cd absorption (Flanagan *et al.*, 1978).

Previous studies by other investigators found that Cd absorption was 8.9 ± 2.0% for volunteers with serum ferritin concentrations of <20 ng/mL, and 2.3 ± 0.3% in volunteers with normal iron stores of >20 ng/mL (Table 1, Absorption) (Berglund *et al.*, 1994; Flanagan *et al.*, 1978). To reduce the possibility that very low Fe status would influence Cd absorption, volunteers with serum ferritins greater than 25 ng/mL were recruited. A correlation analysis showed that ferritin concentrations (Ln serum ferritin) did not significantly correlate with Cd absorption (%) ($P > 0.3$, $R^2 = 0.087$) (Table 7).

The overall apparent absorption of 10.6 ± 4.4% for ^{113}Cd from the labeled SFK was higher than the 2.7% absorption found in crabmeat intrinsically labeled with the radioactive isotope ^{115m}Cd (Newton *et al.*, 1984). However, an extrinsic label of ^{115m}Cd in rolled oats and milk gave values comparable with ours (Table 1, Absorption) (Flanagan *et al.*, 1978; Newton *et al.*, 1984). Please recall that the SFK in the present study were highly processed into a buttery paste. This processing technique could have increased Cd availability for absorption, and led in part to the seemingly high absorption rate of Cd from this food source. In addition, we determined absorption by collecting the feces and measuring the amount of nonradioactive ^{113}Cd by mass spectrometry. This method may be less sensitive than the one used to measure the disappearance of radioactive ^{115m}Cd or ^{109}Cd from the body by doing whole body counting of the isotope or by counting radioactivity in the fecal excretions.

In summary, a very limited number of food Cd values in the GRAND nutrient database that were derived from the FDA Total Diet Study has allowed

TABLE 7

Percentage Cd Absorption as It Relates to Sex, Age, and Serum Ferritin Concentrations

Age (years)	Serum ferritin (ng/mL)	Cd absorption (%)[b]	Measurable fecal ^{113}Cd enrichment[a] (days)
69.8	60.3	18.3	11
65.6	34.4	15.9	5
52.6	85.1	14.5	9
48.0	118.7	14.3	8
53.5	68.1	12.4	4
38.0	30.8	11.4	7
59.5	37.5	10.4	3
30.8	40.2	10.3	8
60.7	126.7	10.2	6
38.1	27.4	9.1	11
34.4	51.8	8.6	7
47.8	37.0	7.5	7
57.1	151.6	4.7	4
68.6	151.7	1.6	5
Mean ± SD 52 ± 13	73 ± 46	10.6 ± 4.4	6.8 ± 2.5

[a]Number of days on which ^{113}Cd enrichment could be accurately detected in the feces.

[b]Apparent fractional absorption of Cd was calculated by subtracting the amount of ^{113}Cd excreted in the feces, in excess of natural abundance, from the amount fed in the breakfast meal. This amount was then divided by the amount of ^{113}Cd in the meal.

TABLE 8

Nutrient Composition of Self-Selected Diets Based on a 3-Day Food Diary and Analyzed with the GRAND Database

Nutrients	Calculated[a] (units/day)	RDA or DRI[b] (%)
n	14	—[c]
Calories, kcal	1930 ± 506[e]	—
Protein, g	75 ± 22	150
Fat, g	75 ± 28	—
CHO, g[d]	243 ± 74	137
Calcium, mg	760 ± 354	63
Cadmium, μg	14.4 ± 5.8	—
Copper, mg	1.2 ± 0.5	50[e]
Iron, mg	16 ± 6	160
Zinc, mg	11 ± 4	92
Vitamin C, mg	130 ± 75	217

[a]Mean ± SD.

[b]Recommended Dietary Allowance or Dietary Reference Intakes. Calculated values are expressed as a percentage of the RDA or DRI. The DRI for Ca is 17200 mg.

[c]Indicates a blank space.

[d]Total carbohydrate.

[e]Estimated Safe and Adequate Daily Dietary Intake.

us to closely predict fecal Cd output (2.1% error) for this small study population that consumed a typical Western mixed-food diet. The study showed that the apparent absorption of ^{113}Cd incorporated into SFK and determined by IDICPMS, was approximately 10% of intake in women volunteers. However, this amount of absorption might represent an upper limit because highly processed kernels were used. Digestion of the processed kernels in the intestinal track might have been more complete with better release of Cd compared with that from whole kernels. Nonetheless, the results suggest that the availability of Cd from the highly processed SFK to humans is similar to that reported for other types of foods. Whether this level of Cd absorption could be a significant biological problem for humans is not yet known. However, a recent study in our laboratory (Reeves *et al.*, 2001) showed no significant changes in the concentrations of Cd in red blood cells, hair, or urine in women volunteers who consumed 255 g of sunflower kernels per week for 48 weeks. The SFK provided approximately 130 μg Cd/week, in addition to that found in the normal mixed-food diet, but the SFK were consumed as the whole kernel rather than the highly processed sunbutter.

ACKNOWLEDGMENTS

The authors thank Lorna Hammerlund and Deb Christopherson for their valuable laboratory assistance. We also greatly appreciate the cooperative spirit that each of the volunteers brought to the study. Without their positive attitudes and dedication, this study would not have been possible.

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